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Identify contaminants with decontam on the QIIME 2 Framework

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ABSTRACT Here, we present the integration of the decontam method for contaminant identification and a supplemental approach for identifying the source of contaminants in sequencing data within the QIIME 2 Framework for microbiome data science. We demonstrate its use in a tutorial based on the QIIME 2 "Moving Pictures Tutorial" data.

KEYWORDS microbiome, contamination, decontam, QIIME 2, amplicon, metagenome

Contaminant identification, within a sequenced-based experiment, is crucial to an analysis as contaminants can affect scientific interpretations and downstream processes (1–5). While much work has been done in this area (6–8), the use of these techniques has not been consistently implemented. To this end, here, we expanded the contaminant identification options available within the QIIME 2 Framework by incorporating decontam, an established bioinformatic method for identifying contaminants in taxonomic feature tables derived from microbiome sequencing data (either amplicon or metagenome) (7) previously only available as an R package. QIIME 2 is a very widely used platform for microbiome data analysis (9–13). The QIIME 2 Framework (Q2F, currently being rebranded as *rachis*) is a Python-based framework that works on the basis of "plugins" or software packages that ease integration between diverse analysis methods (14). The plugin mechanism has been used to compose prominent bioinformatics software packages from other developers such as DADA2 (15), Deblur (16), and Kraken2 (17). While dedicated contaminant identification methods have not been available within QIIME 2 until recently, with the addition of SCRuB (8), the decontam functionality, is now implemented within the QIIME 2 q2-quality-control plugin (https://github.com/qiime2/q2-quality-control), which is provided by default in both the QIIME 2 and MOSHPIT (18) (i.e., the amplicon and metagenome suites of tools, respectively) distributions. This functionality is, therefore, available for Linux, macOS, and Windows (via WSL) and is available in the Docker container builds provided for all QIIME 2 and MOSHPIT releases. Installation instructions can be found at https://library.qiime2.org/quickstart.

Three new QIIME 2 actions were implemented—decontam-identify, decontam-score-viz, and decontam-identify-batches. The decontam-identify action, written in Python and R, implements the core decontam functionality of assigning scores to taxonomic features, indicating their consistency with contaminant or non-contaminant origin (7). The decontam-score-viz action—utilizing Python, HTML, CSS, and JavaScript—produces as output a histogram of the decontam scores and a table of taxonomic features along with their decontam scores, abundances, prevalences, and classifications as contaminant or non-contaminant. If representative sequences were provided, the table includes hyperlinks to a web-based BLAST search of those sequences against NCBI's "core_nt" reference database (Fig. 1b and c). The decontam-identify-batches action is a QIIME 2 pipeline action intended for identification of batch-associated contamination and can aid users in localizing contaminants to specific sections of their measurement protocol such as sequencing run, kit, or date of extraction.

All technical information associated with these actions can be seen in Table 1. To demonstrate the use of the new decontam functionality within QIIME 2, a tutorial was

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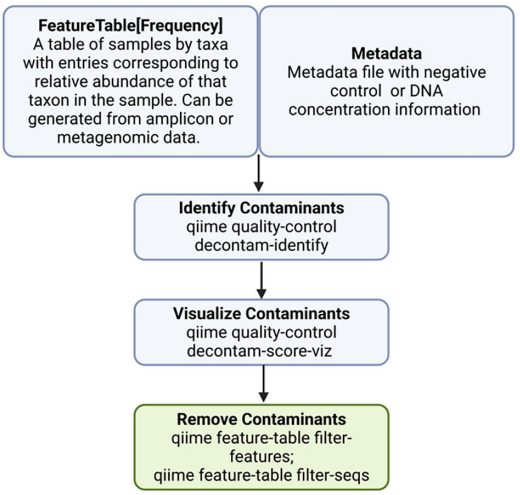
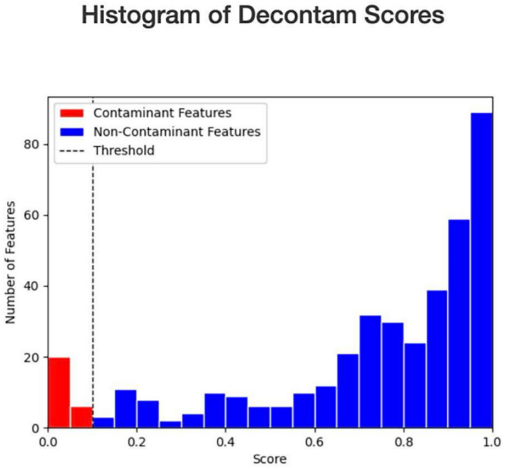
a.



b.



* 369 Non-Contaminant Features were assigned a score of NA (not plotted)

Contaminant Features	Non-Contaminant Features	Contaminant Features %
26	744	3.38

c.

Click on a Column header to sort the table.

Feature ID	Determination	P-Score	Abundance	Prevalence	Sequence
4b5eeb300368260019c1fbc7a3c718fc	Non-Contaminant	0.76	11373	13	TACGGAGGATCCGAGCGTTATCCGGATTTATTGGGTTTAAAGGGAGCG
fe30ff0f71a38a39cf1717ec2be3a2fc	Non-Contaminant	0.83	8929	16	TACGTAGGGTGCGAGCGTTAATCGGAATTACTGGGCGTAAAGCGAGCG
d29fe3c70564fc0f69f2c03e0d1e5561	Contaminant	0.07	8622	25	TACGTAGGTCCCGAGCGTTGTCCGGATTTATTGGGCGTAAAGCGAGCG
868528ca947bc57b69ffdf83e6b73bae	Non-Contaminant	0.52	7663	10	TACGGAGGATCCGAGCGTTATCCGGATTTATTGGGTTTAAAGGGAGCG
154709e160e8cada6bfb21115acc80f5	Non-Contaminant	0.81	7412	13	TACGGAGGATCCGAGCGTTATCCGGATTTATTGGGTTTAAAGGGAGCG
1d2e5f3444ca750c85302ceee2473331	Contaminant	0.03	7186	23	TACGGAGGGTGCGAGCGTTAATCGGAATAACTGGGCGTAAAGGGCACG
0305a4993ecf2d8ef4149fdfc7592603	Non-Contaminant	0.85	5390	11	TACGGAGGATCCGAGCGTTATCCGGATTTATTGGGTTTAAAGGGAGCG
cb2fe0146e2fbcb101050edb996a0ee2	Non-Contaminant	0.96	4651	16	TACGTAGGTGGCAAGCGTTATCCGGAATTATTGGGCGTAAAGCGCGCG
997056ba80681bbbdd5d09aa591eadc0	Contaminant	0.0	3950	16	TACGTATGTCACAAGCGTTATCCGGATTTATTGGGCGTAAAGCGCGTC
3c9c437f27aca05f8db167cd080ff1ec	Contaminant	0.0	3023	14	TACGGAAGGTCCAGGCGTTATCCGGATTTATTGGGTTTAAAGGGAGCG

FIG 1 QIIME 2 decontam workflow and example output. (a) Standard workflow for using decontam within the QIIME 2 Framework with required inputs. To remove identified contaminants, the actions "qiime feature-table filter-seqs" and "qiime feature-table filter-features" are utilized. (b) Histogram of decontam scores produced as output from decontam-score-viz. (c) Table of taxonomic features and associated information output by decontam-score-viz.

TABLE 1 Technical information for the actions implemented within the QIIME 2 framework

		Flag	Qiime 2 data type	Description
decontam-identify	Inputs	--i-table	Feature Table [Frequency]	Feature table from which contaminated sequences will be identified from
		--m-metadata-file	METADATA	Metadata file indicating which samples in the experiment are control samples
	Outputs	--o-decontam-scores	FeatureData [DecontamScore]	The resulting table of scores from the decontam algorithm that scores each feature on how likely they are to be a contaminant sequence
decontam-score-viz	Inputs	--i-table	FeatureTable [Frequency]	Feature table from which contaminated sequences will be identified from
		--o-decontam-scores	FeatureData [DecontamScore]	The resulting table of scores from the decontam algorithm that scores each feature on how likely they are to be a contaminant sequence
	Outputs	--o-visualization	VISUALIZATION	Visualization to be rendered via QIIME 2 View
decontam-identify-batches	Inputs	--i-table	Feature Table [Frequency]	Feature table from which contaminated sequences will be identified from
		--m-metadata-file	METADATA	Metadata file indicating which samples in the experiment are control samples
	Outputs	--o-batch-subset-tables	Collection [FeatureTable [Frequency]]	Directory where feature tables split based on metadata and parameter split-column values
		--o-decontam-scores	Collection [FeatureData [DecontamScore]]	The resulting tables of scores from the decontam algorithm that scores each feature on how likely they are to be a contaminant sequence
		--o-score-histograms	VISUALIZATION	Visualization for all data subsets; to be rendered via QIIME 2 View

generated using the QIIME 2 Moving Pictures Tutorial data (19) available at https://amplicon-docs.qiime2.org/en/latest/how-to-guides/decontam. This software is made available under the BSD 3-Clause license, and the tutorial is available under the CC-BY license.

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DATA AVAILABILITY

Example data sets used for validation and tutorial generation can be found at "https://data.qiime2.org/2024.10/tutorials/moving-pictures/emp-single-end-sequences/sequences.fastq.gz" as part of the QIIME 2 "Moving Pictures Tutorial." These data are a subset of the sequence data and sample metadata that are publicly available under the "Moving Pictures of the Human Microbiome" project [MG-RAST:4457768.3-4459735.3].

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